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A Test for Synergism Between DDT and Nicotine-Bentonite in Dusts

NEELY TURNER, *Conn. Agr. Expt. Sta., New Haven* and D. H. SAUNDERS, *Eastern Regional Research Laboratory, U.S.D.A., Wyndmoor, Pa*

Synergism has been defined by Macht (1929) as "the phenomenon exhibited by the combination of two or more drugs in which the pharmaco-dynamic effect produced by the mixture is not a simple summation of the effects produced by the two or more individual components." Horsfall (1945) has discussed synergism and antagonism fully and has reviewed methods of studying the phenomenon and measuring its magnitude. He suggests the use of mixtures of the chemicals in various proportions, a system used by Le Pelley & Sullivan (1936) and expanded by Dimond & Horsfall (1944).

Interest in the possibility of synergism between nicotine and DDT led to a study of the two materials applied as dusts to control the European corn borer, *Pyrausta nubilalis* Hubn., (Woodward *et al.* 1946).

MATERIALS.—The dust samples were prepared by the Eastern Regional Research Laboratory from technical DDT and commercial *Black Leaf 155* (13.8 per cent nicotine by analysis) used as the source of nicotine bentonite. *Pyrax ABB* was used as the pyrophyllite diluent.

Samples 1 through 4 were prepared by mixing the pyrophyllite and the amount of nicotine bentonite required to give the desired nicotine content in the dust mixture and ball milling for a period of 30 minutes.

In the case of samples 5 through 8 the required amount of DDT was dissolved in acetone and mixed with pyrophyllite using sufficient solvent for thorough moistening. The mixture was freed of all solvent by drying in air at room temperature. It was then ball milled for a period of 30 minutes.

Samples 9 through 20 containing both fixed nicotine and DDT were prepared by solvent depositing the DDT on pyrophyllite as in samples 5 through 8 and mixing the dried product with the required amount of nicotine bentonite in the ball mill for a period of 30 minutes. All of the above 20 samples were dustable and hence were not sieved.

Samples 21 and 22 were prepared by the method used for samples 5 through 8 except that *Volclay* bentonite was used as the diluent and it was necessary to powder the mixture by a painful process of grinding with a mortar and pestle. The product was screened through an eighty mesh screen which was as fine a dust as conveniently was feasible. Ball milling was not possible due to agglomeration of the material in the mill. These last two dusts were heavier and had poorer dustability as compared with previous samples.

Table 1.—Control of European corn borer using nicotine bentonite dusts, DDT dusts and mixtures of the two.

Material No.	Per Cent Nicotine	Per Cent DDT	No. Larvae	Reduction in Larvae
1	1.0	0.0	65	39.8%
2	2.0	0.0	60	44.4
3	4.0	0.0	36	66.7
4	8.0	0.0	31	71.3
5	0.0	1.0	32	70.4
6	0.0	2.0	5	95.4
7	0.0	4.0	18	83.3
8	0.0	8.0	3	97.2
9	.75	.25	46	57.4
10	1.5	.5	29	73.1
11	3.0	1.0	24	77.8
12	6.0	2.0	12	88.9
13	.5	.5	30	72.2
14	1.0	1.0	33	69.4
15	2.0	2.0	26	75.9
16	4.0	4.0	15	86.1
17	.25	.75	36	66.7
18	.5	1.5	39	63.9
19	1.0	3.0	12	88.9
20	2.0	6.0	15	86.1
21	0.0	4.0	9	91.7
22	0.0	8.0	16	85.2
23	None	None	113	—
24	None	None	102	—
	Mean of 23 and 24		108	

Methods.—Lincoln sweet corn was planted June 19 for infestation by the second generation of the European corn borer. Plots were two rows wide and ten feet long, and were randomized in three blocks, with two untreated checks in each block. The corn was in the early green tassel stage when the first treatment was applied on August 8. A small hand duster was used, with application to the tassels only. Further treatments were applied on August 15, 20 and 26 to the developing ear shoots. The infestation following treatment was determined by dissecting ten stalks taken at random from each plot. Dissections were made early in September when the corn was in the roasting-ear stage.

Results.—A summary of the results of the dissection is given in table 1. As is usually the case when the infestation of corn borers is light, there was great variation in the results. However, since four concentrations of each mixture were used, reasonably satisfactory dosage-response curves can be drawn. Curves based on

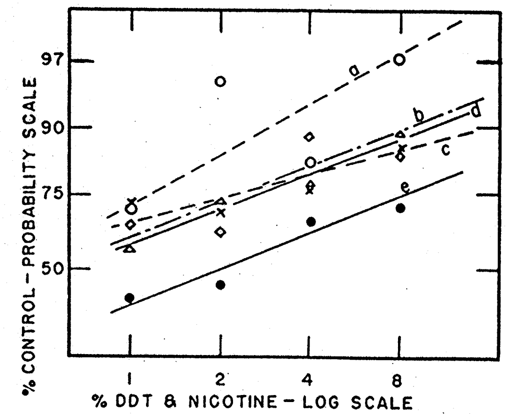

Fig. 1.—Dosage-response curves based on DDT content only. (a) DDT only, (b) equal parts DDT and nicotine, (c) 3 parts DDT and 1 part nicotine and (d) 1 part DDT and 3 parts nicotine.

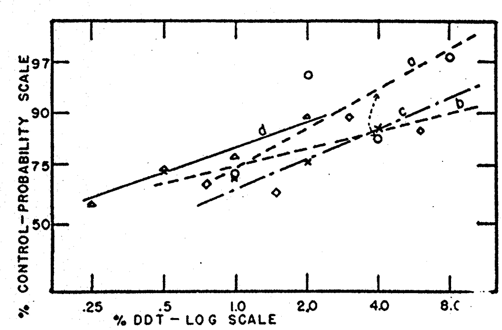

Fig. 2.—Dosage response curves based on total percentages of DDT and nicotine. (a) DDT alone, (b) 1 part nicotine and 3 parts DDT, (c) equal parts nicotine and DDT, (d) 3 parts nicotine and 1 part DDT and (e) nicotine alone.

the total percentage of toxicant are shown in figure 1. It is evident that nicotine-bentonite was much less effective than DDT. It is also evident that the mixtures were intermediate in toxicity. However, their position is obviously not determined entirely by the proportion of either toxicant. The curve for the 25 per cent DDT and 75 per cent nicotine mixture was displaced to the left in relation to the other mixtures—it was more toxic than its composition indicated.

Since DDT was the more toxic material, curves were drawn on the basis of DDT content only (Fig. 2). In spite of the heterogeneity, it is evident that some of the mixtures were less toxic than would be expected from the DDT content alone. These were all concentrations of the 75 per cent DDT-25 per cent nicotine, and three of the four concentrations of the 50 per cent DDT-50 per cent nicotine mixture. Obviously the nicotine was actually antagonistic to the DDT. The 25 per cent DDT-75 per cent nicotine mixture was consistently more toxic than would be expected on the basis of the DDT alone. It was the only combination that showed any evidence for synergism.

Table 2.—Calculation of data in the test for synergism as suggested by Wadley (1945).

Material No.	Per Cent Nicotine	Per Cent DDT	DDT Equivalent	Mortality Observed	Mortality Calculated	Synergism
9	.75	.25	.33	58	56	+
10	1.5	.5	.66	71	69	+
11	3.0	1.0	1.32	82	81	+
12	6.0	2.0	2.64	90	89	+
13	.5	.5	.55	68	66	+
14	1.0	1.0	1.11	74	78	−
15	2.0	2.0	2.21	80	87	−
16	4.0	4.0	4.43	86	93	−
17	.25	.75	.78	60	73	−
18	.5	1.5	1.55	74	83	−
19	1.0	3.0	3.11	85	91	−
20	2.0	6.0	6.21	92	95	−

DDT equivalent at L.D. 50 = .111
L.D. 90 = .102
Mean value = .107

The data have been examined by the method of Wadley (1945). If it is assumed that the curves for DDT alone and for nicotine bentonite are parallel, the nicotine equivalents can be calculated and compared with the observed values. Since the equivalent value is determined from the curves, it is logical to interpolate the "observed" values from figure 1 rather than to use the mortalities in table 1. Results of the calculation are given in table 2, and show a slightly higher observed than calculated value in all concentrations of the three parts nicotine and one part DDT mixture and in the lowest concentration of the 2-2 mixture. These differences are not significant statistically.

Horsfall (1945) has described a graphic method of examining data for synergism. He has suggested that the points for the two ingredients used alone be connected by a straight line, and that the values as observed be plotted and connected. Any bulge above the "expected" straight line may represent synergism. The data have been so plotted in figure 3, using mortalities interpolated from the curves in figure 1. Observed values greater than expected occurred in the same materials that showed the same relationship according to Wadley's method. Again, however, the differences are relatively slight.

Following the method of Dimond & Horsfall (1944), the dosage required to kill 80 per cent of the corn borers has been determined from the curves in figure 1. The calculations show (Table 3) that as the proportion of nicotine was increased,

Table 3.—Concentrations of DDT and nicotine alone and in mixtures required for equal control.

Proportion of Ingredients		Concentration Required for 80% Control		
DDT	Nicotine	Total	DDT	Nicotine
100	0	1.4	1.4	0
75	25	2.8	2.1	.7
50	50	3.5	1.75	1.75
25	75	4.0	1.0	3.0
0	100	13.0	0	13.0

the total percentage of nicotine and DDT required was also increased. Only the mixture of one part DDT and three parts nicotine required less DDT than DDT used alone. In this mixture the three per cent of nicotine replaced only .4 per cent of DDT as determined by the difference between the 1 per cent of DDT used and the 1.4 per cent necessary to control 80 per cent when no nicotine was added.

Discussion.—It is obvious that antagonism occurred between nicotine and DDT when mixed in certain proportions. While synergism may have occurred in other mixtures, it was too small to be measured by this test. Both of the graphical methods showed evidences of synergism of identical mixtures. Horsfall's (1945) method assumes that combinations of the two materials will fit a straight line drawn between the points plotted for the two chemicals used alone if neither synergism nor antagonism occur. This is certainly correct if the dosage-response curves of the mixtures of the two materials are parallel. Wadley's method assumes that all of the curves are parallel. In the

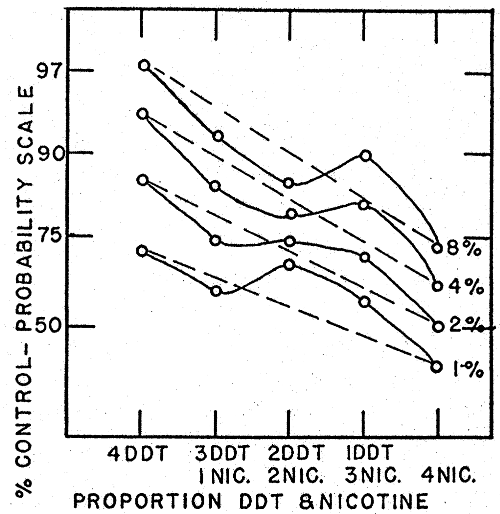

Fig. 3.—Test for synergism by the method of Horsfall (1945). Broken lines show the "expected" mortality and solid lines the observed mortality.

case of the DDT-nicotine mixtures the curves appear to have different slopes, but the heterogeneity is too great for statistical proof.

It is certainly probable that one of the evidences of synergism may be a change in slope of the dosage-response curves. It also seems possible that combination of two materials which have non-parallel dosage-response curves might produce a change in slope, whether or not synergism occurred.

The data give no clue as to the reasons for the antagonism noted. The two dusts containing only DDT and a bentonite diluent performed about as well as the DDT-pryophyllite dusts. The DDT-bentonite dusts had very poor physical properties for dusting and could not be applied uniformly with the equipment used. Certainly the relatively smaller quantities of bentonite in the dusts containing nicotine-bentonite should not be responsible for the antagonism. Furthermore, the one series which was not antagonistic contained the largest proportion of nicotine-bentonite.

The lack of evidence of synergism found in some mixtures of DDT and nicotine-bentonite dusts in these tests should not be interpreted to mean that no synergism may occur between other forms of nicotine and DDT used in different ways, as shown by Woodward *et al.* (1946).

Summary.—The possibility of synergism between DDT and nicotine-bentonite in dusts was studied using the European corn borer as the test insect. The method used was application of the two materials together with mixtures in 3 to 1, 2 to 2 and 1 to 3 ratios.

Mixtures of three parts DDT and one part nicotine showed some evidences of antagonism, as did three of the four concentrations of the 2–2 mixture.

One concentration of 2–2 mixture and all of one part DDT and three parts nicotine showed slight evidences of synergism not statistically significant by the graphic methods of Horsfall and of Wadley. The increased mortality observed was too small in terms of material required to control the European corn borer to merit further study.—2-10-47.

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